PATENT
45D-1750 (81841.0041)

#30 Linda

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

R. J. Obremski, *et al.*

Serial No: 09/063,978

Filed: April 21, 1998

For: DETECTION OF VERY LOW QUANTITIES OF ANALYTE BOUND TO A SOLID PHASE

Art Unit: 1645

Examiner: J. Hines

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, Washington D.C. 20231, on
5/13/2002
Date of Deposit
Wei-Ning Yang, Reg. No. 38,690
Name
Signature 5/13/02 Date

DECLARATION UNDER 37 C.F.R. § 1.321

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, John Silzel, Ph.D., declare as follows:

1. I am a co-inventor named in the above-identified patent application.

2. I have read and understood the specification of the application, the Advisory Action dated December 13, 2001, and the references cited by the Examiner, including EP 304,202 (the '202 patent); Ekins *et al.*, *Analytica Chimica Acta* ("Analytica reference"), and Ekins *et al.*, *Journal of Clinical Immunoassay* reference ("Immunoassay reference"). I am making this Declaration in support of my opinion that in view of the cited references, it was unexpected by those skilled in the art at the time the invention was made that an analyte contained in a liquid sample can be substantially depleted by and concentrated onto microscopic sorbent zones.

3. The Examiner appears to believe that it would have been obvious to use the technique of allowing for analyte depletion in a sample as taught by Figure 4 of the Immunoassay reference in the binding assay of the '202 patent that teaches using small amounts of binding agents. The Examiner further argues that because the '202 patent recognizes the use of small amounts of the binding agents, it would be feasible to place the binding agent on a very small area of a solid support because "[a] high coating density is generally desirable to maximize signal/noise ratios." I disagree with this conclusion.

4. As I will explain in more detail below, Figure 4 does not teach or suggest a substantial depletion of an analyte by and concentration onto microscopic sorbent zones. Also, it is my opinion that the ability of the present invention to concentrate the binding capacity of a conventional microplate well onto an area of a binding zone that is about 100 times smaller without a significant loss of analyte-depletion efficiency was unexpected by those skilled in the art. In addition, it was also not expected in the art that microscopic sorbent zones in contact with conventional sample volumes are capable of harvesting substantially the same amount of the analyte from the sample within the same incubation time as the equivalent amount of the antibody spread across a microplate well or distributed in solution. Furthermore, I will demonstrate that based on the novel and the unexpected approach of the present invention, we were able to achieve substantial advantages over conventional microplate assays.

5. The present invention relates to a microscale-binding assay, an analyte-binding array, and a kit for use in a mass-sensing binding assay with a high sensitivity for very low quantities of analyte. All three independent claims 1, 23, and 26 require: a microscopic size of the sorbent zones, a substantial depletion of analyte from the sample, and a concentration of the depleted analyte on the microscopic sorbent zones. These three limitations are crucial for the successful implementation of the mass-sensing assay of the present invention.

6. Figure 4 of the Immunoassay reference does not teach or suggest that analyte contained in a liquid sample can be substantially depleted by and concentrated onto microscopic sorbent zones. All that Figure 4 demonstrates is a theoretical capability of an immunosorbent phase to capture nearly 100% of the antigen when an antibody concentration is ten times greater than the affinity constant. The plot also shows the opposite extreme of having the antibody present at low concentrations (one tenth the affinity constant). These types of curves are analogous to theoretical titration curves known in the art and do not, in themselves, convey any new information, just that theoretically given enough antibody and enough time, one can bind a certain amount of analyte.

Furthermore, Figure 4 of the Immunoassay reference shows a theoretical depletion of an analyte from the solution under equilibrium conditions. In fact, the recent article by Sapsford *et al.* (*Anal. Chem.* (2001) 73, 5518-5524) reviews Etkins'

ambient analyte assay and states that Etkins' ambient analyte theory and calculations require an assumption of an equilibrium or static conditions (page 5523, left column, first paragraph). Such assumptions may be appropriate to model an ambient analyte assay of Ekins, which may be approximated as a dilute antibody solution and a simple first-order non-cooperative binding because so few binding events take place (the bulk concentration of the analyte is not perturbed). However, when a system deviates from an ideal dilute homogeneous liquid solution, as in the assay of the present invention in which the analyte is depleted from the solution, such an approximation is not acceptable.

Figure 4 of the Immunoassay reference assumes that the concentration of the binding partner can be defined as the mass of the binding partner divided by the volume of the solution. This simplification predicts the same equilibrium results regardless of whether the binding partner is in solution, immobilized in monolayer fashion on the walls of the vessel, or confined in multiple layers to a tiny sorption zone. The simplification also neglects the potential for immobilized binding partner molecules to "block" underlying layers of the binding partner when the immobilization is done at a high concentration of the binding partner and multiple layers of the binding partner are formed. The simplification also neglects the multiple valency of the antibody, assumes a first-order binding process, neglects the heterogeneity of the antibody-binding affinities, assumes equilibrium has been reached, and neglects the effect of immobilization on the antibody affinity.

In summary, the mass action law model shown in Figure 4 of the Immunoassay reference is purely theoretical. It assumes equilibrium conditions and simple first-order binding. Thus, it is not suitable for modeling a complicated non-first order binding process of our invention. Therefore, Figure 4 does not teach or suggest a substantial depletion of an analyte by and concentration onto microscopic sorbent zones.

7. Although analyte depletion from a sample by wells of microplates is generally known in the art, in my view, the ability of the present invention to concentrate the binding capacity of a conventional microplate well onto an area of a binding zone that is about 100 times smaller without a significant loss of analyte-depletion efficiency was unexpected by those skilled in the art. I believe that prior to the present invention, it was generally understood in the art that when multiple layers of the binding partner are immobilized on a surface, the overall binding capacity of the binding partner substantially decreases due to "blocking" of the lower layers by the top layers.

My view on the state of the art prior to the present invention is supported by the attached article from Analytical Biochemistry, which reviews the state of the microarray hybridization art prior to 2001 (B. A. Stillman and J.L. Tonkinson, *Analytical Biochemistry* (2001) 295(2):149-157). The article states that microarrays constructed on two-dimensional surfaces have been thought in the art to allow a

greater access of the analyte to the binding agent resulting in a more efficient hybridization and a stronger signal as compared to three-dimensional surfaces (see the last paragraph on page 149 and the first paragraph on page 160). Furthermore, the article concludes that "[i]n contrast to earlier reports, [their] data indicate that DNA noncovalently immobilized on a two-dimensional surface interact[s] with solution-phase species in a manner remarkably similar to DNA noncovalently immobilized on a three-dimensional microporous surface" (*see* page 150, left column, last paragraph). Thus, the article indicates that, at least prior to 2001, those skilled in the art were discouraged from using three-dimensional multi-layer matrixes of the binding partners for analyte binding.

In the present invention, however, about 10^{10} molecules of binding partner are immobilized per each sorbent zone (*see* Example 1 of the present invention) resulting in a three-dimensional matrix about 3 monolayers deep (*see*, for example, page 19, lines 16-31, of the instant specification discussing irregular topology of the immobilized binding-partner molecules extending up to 200 nm vertically from the surface of the film). As discussed above, in my view, those skilled in the art would not have expected that such a three-dimensional structure is possible without "blocking" the analyte access to the binding partner molecules on the lower layers. However, we were able to achieve functionality and accessibility of the binding partner throughout the three-dimensional matrix by using a photo-linking technique and printing the antibody at concentrations 1000 times greater than the

1 μM solutions typically used to coat microplates (page 17, lines 18-32 of the specification).

Therefore, I believe that it was unexpected by those skilled in the art that a three-dimensional cross-linked matrix of the binding-partner molecules of the present invention (page 19, lines 16-31, of the specification) allows the same level of analyte binding as the two-dimensional surface of a microplate well.

8. Finally, I believe that, prior to the instant invention, it was not expected in the art that microscopic sorbent zones in contact with conventional sample volumes are capable of harvesting substantially the same amount of the analyte from the sample within the same incubation time as the equivalent amount of the antibody spread across a microplate well or distributed in solution. Such a result was unexpected given the complexities of nonequilibrium conditions, cooperativity in binding, diffusion, local concentration effects, and other nonlinear effects occurring with sorption on microscopic sorbent zones.

Indeed, the above article by Sapsford *et al.* demonstrates a complicated and unpredictable nature of analyte binding by microscopic sorbent zones. The article demonstrates that mechanisms involved in such binding are not well understood and are often disputed by various research groups. The article reports results that are contrary to the teachings of Ekins. For example, it states, "[t]he results presented in [the] paper are contrary to the theory discussed by Ekins, which

suggests that the smaller the spot size, the greater the association rate constant should become" (page 5522, right column, last paragraph).

9. Based on a novel and the unexpected approach of the present invention as described above, we were able to achieve substantial advantages over conventional microplate assays. In the microscale assay of the present invention, substantially the same signal as in the conventional microplate assay is generated in a zone only a few hundred microns in diameter, and, thus, having a surface area about 100 times smaller than that of a microwell. Since background signals are roughly proportional to the viewed surface area, the microscale assay of the present invention advantageously produces a signal-to-background ratio that are orders of magnitude greater than in conventional microplate assays. It should be appreciated that the phenomenon of analyte depletion is necessary but not sufficient to realize this advantage. The present invention requires both the depletion of the analyte from the solution and its concentration onto the microscopic sorbent zones.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful

false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: MAY 10, 2002

By: _____
John Silzel, Ph.D.